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Development of NK Humanized Mouse Models for the *In Vivo* Evaluation of NK Cell-Based Therapeutics

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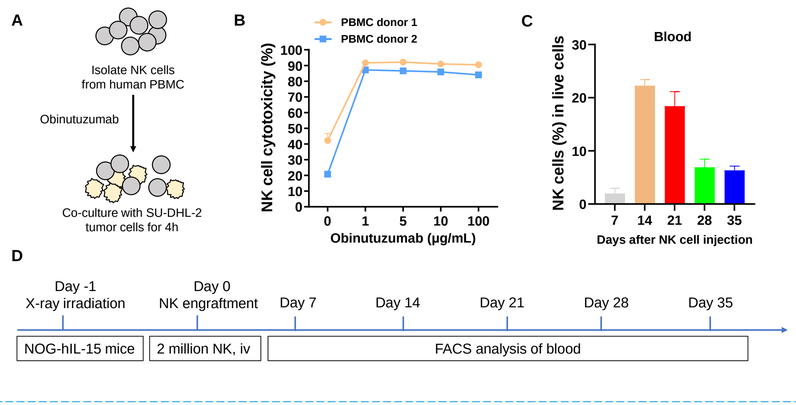
Abstract

With recent advancements (e.g., T/NK/myeloid engagers) in anti-tumor immunotherapy, novel treatments have emerged and progressed rapidly. Several strategies are now being employed to enhance the efficacy of natural killer (NK) cells in tumor immunotherapy, including the use of monoclonal and multi-specific antibodies, as well as immune modulator molecules. Given these developments, there is an urgent need to establish appropriate mouse models to effectively evaluate the therapeutic potential of these emerging immunotherapies.

Methods

In this study, we developed a humanized mouse model for the specific reconstitution of functional and durable human NK cells within a murine system. In this model, human NK cells were purified from peripheral blood mononuclear cells (PBMCs) using magnetic bead separation, expanded *in vitro*, and subsequently engrafted into human IL-15 transgenic NOG mice. We collected blood samples from these mice to monitor human NK cell kinetics and profile using flow cytometry. The therapeutic efficacy of an anti-CD20 antibody was then evaluated in NK-humanized model using the SU-DHL-2 subcutaneous xenograft tumor mode. Additionally, we assessed NK cell intratumoral infiltration and the expression of activation markers by flow cytometry at the end of the *in vivo* efficacy study, providing further insights into the immune responses in the tumor microenvironment.

Results



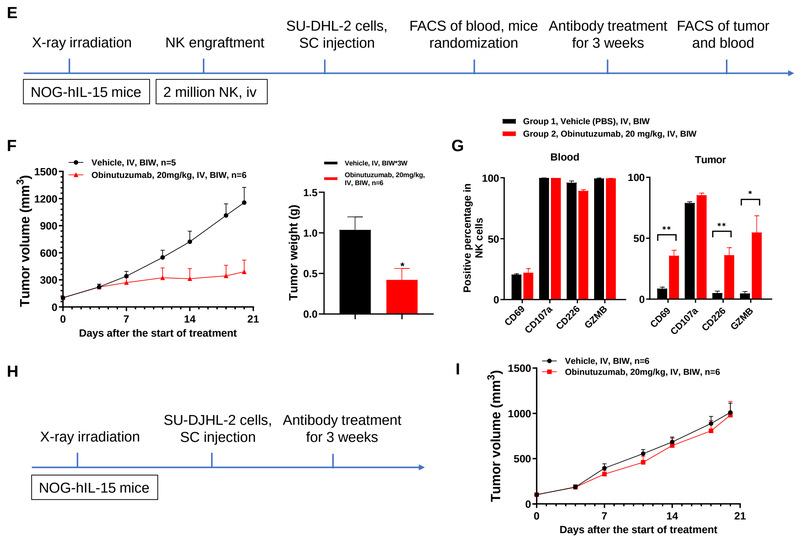
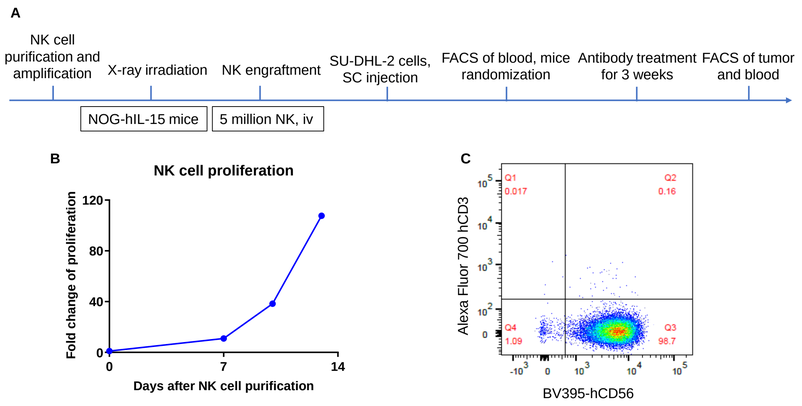


Figure 1. Evaluation of the NK humanized mouse model established with purified human NK cells. (A) Study design of the *in vitro* NK cell cytotoxicity assay. (B) NK cell cytotoxicity detected by flow cytometry. (C) Reconstitution of human NK cells in mice validated by flow cytometry. (D) Timeline for establishing NK cell humanized mice with purified human NK cells. (E) Timeline for *in vivo* evaluation of the ADCC effect of anti-CD20 antibody in NK cell humanized mice. (F) Tumor volume and tumor weight of SU-DHL-2 xenografts treated with anti-CD20 antibody. (G) NK cell activation in blood and tumor detected by flow cytometry. (H) Timeline for *in vivo* evaluation of anti-CD20 antibody in non-NK cell humanized mice. (I) Tumor volume of SU-DHL-2 xenografts treated with anti-CD20 antibody.



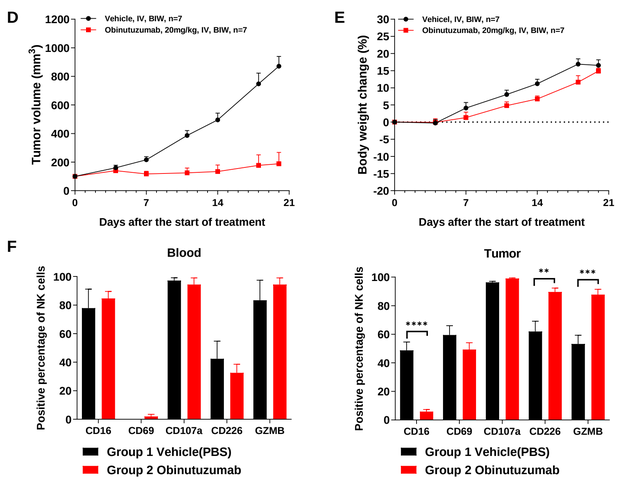


Figure 2. Evaluation of NK cell humanized mice established with amplified human NK cells. (A) Timeline for establishing NK cell humanized mice with amplified human NK cells. (B) Proliferation of purified NK cells over a 2-week period. (C) Purification of amplified NK cells detected by flow cytometry. (D) Tumor volume and (E) body weight of SU-DHL-2 xenografts treated with anti-CD20 antibody. (F) NK cell activation in blood and tumor detected by flow cytometry.

Conclusions

In summary, we developed a humanized mouse model capable of sustaining functional human NK cells for extended periods, ranging from weeks to months. This NK-humanized model provides a valuable preclinical platform for evaluating NK-modulating therapies and may be a promising tool to accelerate the development of novel NK-based immunotherapeutics.

References

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